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Authorizations and Permits for Protected Species (APPS)

File #: 16094
Title: Investigations of Harbor Seals in Alaska
Modification: 6

Applicant Information

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Project Information

File Number: 16094

Application Status: **Application Complete**

Project Title: Investigations of Harbor Seals in Alaska

Project Status: New

Previous Federal or State Permit: 358-1787

Permit Requested:
- MMPA Research/Enhancement permit

Where will activities occur? US Locations including offshore waters

Research Timeframe: **Start:** 01/01/2012 **End:** 12/31/2016

Sampling Season/Project Duration: The Alaska Department of Fish and Game marine mammal program will conduct harbor seal research year round until the end of 2016. Live capture operations will be conducted from February through November each year for up to five years at locations in Southeast Alaska, the Gulf of Alaska, Lake Iliamna and/or the Bering Sea. Aerial surveys, scat collection, sample loans, transfers and import/exports will occur throughout the year for up to five years.

Abstract:

The overall purpose of this research is to provide a greater understanding of the proximate and ultimate factors that regulate the abundance of harbor seals (Phoca vitulina) throughout their range in Alaska, which is required to develop effective management and conservation strategies. Research activities and the maximum number of animals taken per year (n) per activity include: aerial surveys for population census and radio tracking (n=180,000); incidental disturbance during capture activities (n=7,000); ground surveys for photo-identification, counts and behavioral observations (n=10,000); vessel approaches of

animals equipped with bio-logging equipment (n=50 seals; ~5 approaches/seal); vessel surveys for radio tracking and incidental disturbance associated with approaching animals equipped with telemetry equipment (n= 4,000); tissue sample collection from subsistence harvested animals and other mortalities (n=1,750); export of tissue samples for analysis (e.g., prey remains from scat samples) (n=2,000); capture by entanglement in a net in the water or by hoop net or dip net on land (n=350); chemical restraint (n=350); physical restraint by hand, net, cage or stretcher (n=350); collection of biological samples (n=350) including: scat, blood, milk (lactating females), blubber, muscle, skin, muscle, hair, mucus membrane swabs, stomach content subsample, tooth and vibrissae; collection of standard morphometrics and weight (n=350); measurement of blubber via ultrasound (n=350); injection of PIT tags and attachment of flipper identification tags (n=350); attachment of external transmitters and data-loggers (n=150). Incidental take of non-target species may include harbor porpoises (Phocoena phocoena) (n=5) during capture activities. Research activities take place throughout the range of harbor seals in Alaska including Southeast Alaska, Gulf of Alaska and Bering Sea. The requested duration of this permit is five years.

Project Description

Purpose:

Objective 1. Monitor harbor seal population trends in Alaska
Objective 2. Describe the movements, diving behavior, and haulout patterns of harbor seals in Alaska
Objective 3. Determine feeding habits and prey requirements of harbor seals in Alaska
Objective 4: Assess health status and physiology of Alaskan harbor seals
Objective 5. Examine life history parameters of harbor seals in Alaska using photo-identification
Objective 6: Monitor demography, pupping and molting phenology, and population trend at a long-term monitoring site (Tugidak Island)
Objective 7. Determine the genetic structure of harbor seals in Alaska
Objective 8. Assess the impact of disturbance on harbor seals in Alaska
Objective 9. Determine the prevalence of infectious diseases and their impact on harbor seal populations in Alaska
Objective 10. Provide support to studies by other investigators that will lead to better understanding of harbor seals in Alaska and other areas.

The Alaska Department of Fish and Game (ADF&G) has regularly been issued NMFS permits for scientific research on Alaskan marine mammals since 1973. In all cases ADF&G personnel have been primary investigators. Previous permits (#s 34, 124, 194, 249, 349, 370, 584, 770, 771, 965, 1000, 358-1585, and our current permit #358-1787) have been for biological and ecological research studies of harbor seals and other pinniped and cetacean species. Previously, ADF&G harbor seal and ice seal research shared the same permit; however, the complexity of both programs has increased and separate permits should better serve the needs of each program.

Much of our work on harbor seals during the 1990s necessarily focused on developing methodologies and obtaining baseline data on trends in abundance, timing of pupping and molting, important prey species, movements, and genetic structure. Trend routes in Southeast Alaska, Prince William Sound and Kodiak were surveyed on a regular basis, and were expanded into Bristol Bay where little was known about population status (Small et al. 2005). Concurrently, sophisticated statistical procedures were developed and applied to trend survey data to reduce variability introduced by confounding factors such as time-of-day, tide, and date that might mask or bias underlying trends (Adkison et al. 2003, Ver Hoef and Frost 2003). Long-term monitoring at study sites such as Tugidak Island and Nanvak Bay have provided detailed information on the chronology of pupping and molting (Jemison et al. 2006), and how these factors may vary over time, with population status, among sex- and age-groups, and among geographic areas. Diet studies based on examination of scat and stomach contents have indicated seasonal and regional differences in key prey species, which are also reflected by seasonal and regional differences in the fatty acid signatures of seal blubber. Numerous papers have been published (see attached list of publications) reporting on our research conducted under these permits, with multiple other manuscripts from our research group and from our collaborators currently in various stages of analysis and writing.

Extensive studies on the movements and diving behavior of harbor seals in various regions of Alaska utilizing new technology based on satellite telemetry (Hastings et al. 2004, Small et al. 2005, Frost et al. 2006) showed that animals tagged in terrestrial habitat generally exhibited a high degree of fidelity to an area, but also undertake local movements. Genetics studies based on maternally-inherited mitochondrial DNA from seals that use terrestrial habitat revealed a high degree of diversity on a relatively fine scale, suggesting limited dispersal even in the absence of physical barriers (O'Corry-Crowe et al. 2003). More recent studies of harbor seals radio-tagged in glacial habitat indicate that glacial seals have markedly different life-history strategies (Blundell et al. 2011) including different foraging strategies and activity budgets during the breeding season, and seasonal movements in winter that, for some individuals, involved traversing long distances (Womble et al. 2010). Genetic data (Herreman et al. 2009) from Microsatellite DNA, inherited from both parents, along with movement data (Womble et al. 2010), and the disproportionately large numbers of seals pupping and breeding in glacial habitat (Calambokidis et al. 1987, Mathews and Pendleton 2006) suggest that seals may travel from other areas to give birth and breed in glacial habitat, and that pups born in glacial fjords may emigrate to other areas, effectively functioning as a source population. Factors affecting gene flow and stock structure may be profoundly different for seals that

only use terrestrial habitat, compared with those that have access to glacial habitat, thus effective management of harbor seal populations requires a better understanding of seals that use glacial habitat.

Currently, approximately 10-15% of harbor seals in Alaska are estimated to use glacial habitat (Bengston et al. 2007), but those data are obtained during molt surveys. Our tagging data indicate that the majority of seals present in glacial habitat during pupping and breeding season are not present in that habitat during molt surveys, thus it is likely that the importance of glacial habitat to reproductive success in harbor seals in Alaska is underestimated. Glacial fjords are also popular tourist destinations; large numbers of vessels visit these areas during critical life history stages for harbor seals (pupping, breeding, and molt) resulting in disturbance of seals. It is therefore important to understand why, how, and when harbor seals use glacial habitat, and whether increasing vessel traffic and the rapid thinning and retreat of Alaskan glaciers associated with climate change could negatively affect harbor seals beyond the confines of specific glacial inlets.

Although the numbers of total allowable captures per year, per age-class and sex is large on our permit, our sample size for an individual capture trip is not large with respect to what is required for a well-designed study. Often we concurrently conduct identical studies in different habitats or regions to allow comparison without the confounding factor of different years that may have different ecological conditions. Further, although we would like to be able to target different ages and both sexes to answer specific questions and we attempt to schedule our captures to increase our likelihood of capturing specific age groups, we cannot control which seals become entangled in our nets. Beyond the research questions specific to each project conducted during individual capture trips, we also continue our comparison of various health and diet indices across regions relative to population trends. Periodically we conduct comparative analyses of these data that were collected over time from different regions throughout changes in population trends among and within regions. Therefore, a full suite of biological samples is collected from any animal that we capture to augment our long-term database and enable these longitudinal comparisons.

The advances made by ADF&G researchers in the understanding of the ecology, physiology, and population trends of harbor seals in Alaska have paved the way for more focused studies that will lead to a better understanding of habitat and prey requirements, possible effects of anthropogenic activities and climate change, and ultimately of the factors that determine population status. The extensive data on abundance and distribution collected in recent years has been incorporated into GIS databases and modeling is underway to describe habitat requirements. More intensive dietary studies are needed to provide the detail necessary to partition requirements among key prey species, and lead to a better understanding of foraging strategy and trophic interactions. More recent studies of detailed dive behavior are currently underway along with linking activity budgets and foraging strategies with diet and body condition to understand how seals forage in different habitats and whether differences in foraging strategies or foraging efficiency among regions provide insights into differing population trends.

Indeed, the harbor seal would seem to be an ideal candidate for advancing our understanding of the ecological role of pinnipeds. The species is widely distributed in inshore waters, making them accessible, and animals can be captured and handled quite easily. They have been proposed for use as an indicator species for the endangered Cook Inlet beluga whales because harbor seals forage in the same habitat and are exposed to the same contaminants and disease factors. Furthermore, an increased understanding of the importance of glacial habitat to harbor seals may also help in predicting effects of receding sea ice on Arctic pinnipeds that are more logistically challenging to study.

Description: Research activities and the maximum number (n) of animals taken per year per activity include: aerial surveys for population census and radio tracking (n=180,000); incidental disturbance during capture activities (n=7,000); ground surveys for photo-identification, counts and behavioral observations (n=10,000); vessel approaches of animals equipped with bio-logging instruments (n=50); vessel surveys for radio tracking and incidental disturbance associated with approaching animals equipped with telemetry equipment (n= 4,000); tissue sample collection from subsistence harvested animals and other mortalities (n=1,750); export of tissue samples for analysis (e.g., prey remains from scat samples) (n=2,000); capture by entanglement in a net in the water or by hoop net or dip net on land (n=350); chemical restraint (n=350); physical restraint by hand, net, cage or stretcher (n=350); collection of biological samples (n=350) including: scat, blood, milk (lactating females), blubber, muscle, skin, muscle, hair, mucus membrane swabs, stomach content subsample, tooth and vibrissae; collection of standard morphometrics and weight (n=350); measurement of blubber via ultrasound (n=350); injection of PIT tags (n=350); and attachment of flipper identification tags, external transmitters and data-loggers (n=150). Incidental take of non-target species may include harbor porpoises (Phocoena phocoena) (n=5) during capture activities. Research activities take place throughout the range of harbor seals in Alaska including Southeast Alaska, Gulf of Alaska and Bering Sea. The requested duration of this permit is five years.

Methods:

Aerial Surveys: Aerial surveys are conducted for radio-tracking or to assess abundance and population trends throughout Alaska. Surveys are flown along the shoreline and over isolated off-shore reefs that are used as haulouts by seals. Surveys may occur anywhere throughout the distribution of harbor seals in Alaska including Lake Iliamna.

Radio-tracking surveys: may be conducted year-round, but generally occur from May through September from a fixed wing aircraft at an altitude of >400 meters and a cruising speed of 185-200 km/h. During radio-tracking surveys the plane will circle within visual contact, but not directly over a group of seals for up to 15 minutes in order to scan through all of the VHF frequencies deployed in the area. Some radio-tracking surveys involve retrieving floating instrumentation that requires a water landing. Most often, these landings do not take place in the vicinity of hauled-out seals.

Population trend surveys: may be conducted year-round but generally occur in May to early July during the pupping season, and in late July through early Sept. during the molt season, from a fixed wing aircraft at an altitude

of 225-450 meters and a cruising speed of 185-200 km/h. Population trend surveys may include 1-5 passes over a group of seals for the duration of several seconds to several minutes (~5 minutes) depending on how long it takes to photograph all of the seals in the area.

The potential reaction to this activity is that if the plane flies too low it could disturb the seals and they would move from land into the water. All surveys are conducted at an altitude that will minimize disturbance. In the event that seals are disturbed the plane will leave the area as soon as possible to allow the seals to haul out again.

For many years the National Marine Mammal Laboratory has collaborated with ADF&G to conduct population trend surveys and the data is shared by both organizations. Aerial surveys are conducted across the state including Southeast Alaska, Gulf of Alaska and the Bering Sea.

Seal capture trips: Our capture techniques for harbor seals require that we set a net at a site where numerous seals of both sexes and all ages are hauled out; i.e., selective captures for specific sex or age groups is not feasible. Thus, our captures yield a mix of ages, yet seasonal timing of capture trips can influence the age and sex composition of captured individuals. For example, the ratio of females to males hauled out at a site is greater earlier in the annual molt season because females generally molt before males do and spend more time hauled out. Similarly, dependent and/or weaned pups can be captured from late May to early July. The ADF&G harbor seal research program endeavors to address a broad scope of scientific questions with each capture trip, thus we encourage a great deal of collaboration with other agencies and institutions. Over the 5-year time period covered by the permit that we seek with this application our objectives will vary, as will the timing of our captures relative to the specific objectives of each capture trip.

During most capture trips all age cohorts are captured. Biological samples are collected from all age cohorts, but the collection protocol may be altered for some adult females and un-weaned pups (see Table 1 attachment). The standard suite of biological samples that we collect includes blood, blubber, skin, hair, and vibrissae. We also collect standard morphometrics and weight, measure blubber via ultrasound, insert a PIT tag, and attach flipper identification tags. During some capture trips we will attach external transmitters and data-loggers, swab mucus membranes, pull a tooth, collect a muscle sample, and/or subsample stomach contents.

Procedures may include the use of general anesthesia or sedatives, but these will not be administered to an unweaned pup captured without its mother, a lactating female captured without its pup, or a female that is visibly pregnant. Drug administration for procedures is conducted as follows:

• Pulling a tooth for age estimation will only be done under general anesthesia and therefore only on animals that can receive general anesthesia (see above).

• Blubber and muscle samples are only collected from animals that are sedated. This includes unweaned pups captured with their mother, weaned pups, juveniles, sub-adults, adult males, adult females that are not lactating or visibly pregnant, and adult females that are lactating and captured with their pup.

• Blood samples are collected without the use of drugs because many blood analyses are affected by their presence. If drugs will be administered to the animal, blood samples will be collected prior to drug administration.

• Expressed milk may be collected from lactating females, with or without sedation.

• A stomach tube insertion may be conducted on pups to obtain a subsample of stomach content. These pups may be captured with or without their mother.

• Collection of skin, hair, vibrissae, morphometrics, weight, blubber ultrasound, insertion of PIT tag , attachment of flipper tag, mucus swab, and attachment of an external transmitter may be done to all animals with or without sedatives. Drug administration is dependent on the status of the animal; -- drugs will not be administered to an unweaned pup captured without its mother, a lactating female captured without its pup, or a visibly pregnant female.

The specifics of each procedure conducted during a capture trips are as follows:

Capture: To catch seals hauled out on land, investigators in 3 or 4 skiffs will approach the group of seals. When properly positioned, approximately 15-30 meters from the seals, one skiff will deploy a capture net over the stern while motoring at moderate speed in an arc in front of the haulout site. The deployment of the net disturbs the seals and they will all move into the water. Another skiff takes the deployed end of the net to shore while an additional skiff attempts to keep the seals from exiting the area within the arc before the other end of the net is taken to shore. Once the net is set, all skiffs will tend the net. The capture net is approximately 90 meters long and

7 meters deep, and constructed with large diameter multifilament nylon twine in order to minimize the possibility of injury to the seals. The net's lead line is lightweight compared to standard seine nets used for commercial fishing. This feature enables entangled seals to swim to the surface to breathe. In most instances, some seals will avoid the net and some will become entangled. Those that avoid the net will be allowed to swim away without further harassment, while any seals that become entangled will be seined to shore or pulled into a skiff as soon as possible. They will be removed from the capture net and put into hoop nets constructed of soft nylon netting. These are standard techniques that have been used to capture seals in California, Oregon, Alaska and Washington (Jeffries et al. 1993, Small et al. 2005, Frost et al. 2006). Once the seals are in hoop nets they are taken to shore or transferred to the research vessel for processing.

Harbor seal captures in glacial habitat will be conducted using monofilament nets (30-cm stretch mesh, 7.5m deep and 60m long) that are easily managed by two people. Personnel work in small inflatable skiffs and deploy a single net near icebergs where seals are hauled out. Each vessel has two people on board working a net and there are 1-3 skiffs doing captures at a time. Nets are constantly tended from close proximity and manually manipulated every five minutes or less to feel for the weight and movement of captured seals. Entangled seals will be quickly pulled into tending skiffs, removed from the capture net, and placed in hoop nets. A support vessel (e.g. 6.5m Boston Whaler) will transfer seals from the capture skiffs to the research vessel where processing takes place or in some cases processing may occur on the support vessel.

In some instances harbor seals may be caught while hauled out on land or ice or while sleeping afloat at the surface. Such captures will involve sneaking up as close as possible to a seal, then attempting to catch it with a hoop net or long-handled dip net.

Captured seals that were not sedated will be released directly into the water immediately after tagging and sampling. The total time from initial capture until release will vary from 20 minutes to 900 minutes depending upon sampling procedures and the sex and age-class of the seal. At times, due to environmental conditions (glacial ice, currents, wind, weather, availability of a safe anchorage) sampling may occur at a distance from the capture sites. In this case, to ensure the safety of both the crew and the animals, the minimum holding time may be increased. We will do everything we can to ensure that seals are rarely held for >400 minutes and sensitive age-classes will always be processed first (e.g., females with pups, pups, or young animals). However, situations may arise where seals might be held longer if the benefits of additional holding time are greater than the consequences of additional holding time. Harbor seals that have been sedated will be held on board until they show full signs of recovery from the effects of the drugs (e.g., are alert at the approach of researchers and show agitation when gently touched).

In 2010 our average holding time was 267 minutes; this includes transport from the capture site to the processing vessel (could be > 60 minutes, depending on ice conditions), processing (about 20-60 minutes, depending on complexity of processing and temperament of animal), and may include recovery from sedation (up to 180 minutes). When seals are captured in ice, animals are usually captured 1-2 at a time over the span of the day, allowing a steady supply of recently captured seals arriving at the main vessel for processing. Conversely, when seals are captured from terrestrial haul-outs, animals are usually captured all at once. The crew can split into 2-4 processing teams (depending on the experience of the crew) and process animals concurrently. In both ice and terrestrial habitats, when a large number of seals are captured over a short period of time, some seals are held for a period before processing can occur. In this case, the crew determines which animals are highest priority (e.g., adult females and pups), and those animals are processed and released first.

Physical restraint: Seals may be restrained in a mono- or multi-filament nylon net during capture and in a hoop net or plywood pen, post-capture. Seals generally spend 1-5 minutes in a capture net in the water or occasionally, during terrestrial captures, may spend up to 15 minutes in the water if they are entangled in a longer seine net with several other seals and are observed floating on the surface and breathing easily. Once it is pulled into an attending skiff, a seal may spend up to 20 minutes in a net while researchers untangle it from the net. Once removed from the capture net, all seals are moved into individual hoop nets and either taken to shore or to the research vessel for processing. During pre- and post- processing the seals are passively restrained in a hoop net (anchored to a solid structure to prevent the seal from escaping) and/or a plywood pen. During the holding period seals will be left alone with minimal disturbance. In such circumstances seals show little evidence of distress and often fall asleep. They will be carefully observed to make sure there are no signs of distress (e.g., respiration is normal). If the respiration rate appears to be abnormal then the heart rate may be monitored either visually or by physical contact with the animal's chest. Any animals showing signs of hyperthermia will be cooled with water; any animals showing signs of hypothermia will be covered with a blanket or have a heat lamp turned on near them.

During processing, seals may be restrained by a stretcher with nylon straps and/or by a person straddling the seal (without putting weight on it that could restrict breathing), pinning its front flippers by its side to prevent forward movement, and gently restraining the head at the base of the skull to prevent the seal from moving or biting. The person restraining the seal continually monitors respiration by feeling chest expansions and observing nostrils flaring.

Biological Sampling, drug administration, biopsy sampling, external instrument placement and marking: Sampling begins by weighing the animal. The animal, restrained in a hoop net and occasionally a brailer bag for more support, is lifted off the ground and the weight is determined with a digital or spring scale. The brailer bag is 30"x30"x40" in size and made of knotless nylon mesh. It has webbing that wraps around the top circumference, sides and bottom of the bag and forms handles at the top for lifting. The bag is rated to hold 454 kg.

Two or, more commonly, three researchers are involved in collecting biological samples from each animal. When there are three, one person is restraining the animal, another is collecting the biological samples and the third is recording data and handing tools/supplies to the person collecting the samples. When there are only two researchers, the person collecting the samples is also recording the data. Once the animal has been removed from its pen or hoop net and properly restrained by a stretcher and/or person the processor starts with the blood draw.

Approximately 90-125 cc of blood (NTE 7.5% of the circulating blood volume) will be drawn from the extradural intervertebral vein (Geraci and Smith 1975) using 1" to 3.5" 18 or 20 gauge spinal needle and put into blood collection tubes. Blood is used for a variety of analyses (e.g., health, disease, and contaminants assessment, diet determination via stable isotope analysis).

The animal is then given an injection of a sedative, (e.g. diazepam at 0.25 mg/kg body weight) in the epidural space using the same needle/injection site as the initial blood draw. The animal will be lightly sedated for 30 - 45 minutes, and continuous attention will be paid to respiration. This technique is based on the methods presented in Walker and Bowen (1993) and Coltman et al. (1998). Sedatives will not be administered to visibly pregnant seals or seals that need to be released immediately (e.g., unweaned pups captured without their mother). Next, to control pain, an intramuscular injection of an analgesic (e.g. Flunixin megulumine at 1-2 mg/kg body weight) is given near the hip (longissmus dorsi). Finally, when available, a sedative reversal drug (e.g. Flumazenil 0.005 to 0.01 mg/kg body weight) is injected into the muscle near the hip (longissmus dorsi) to reverse the Diazepam and minimize the amount of time the animal must be held before release. It is possible that if the animal moves suddenly or if the blubber layer is thicker than expected the IM injection will end up being injected into the fat and by definition be a subcutaneous (SQ) injection. Sedatives, analgesics and reversal drugs will only be administered by trained personnel (see Table 2 attachment).

If General anesthesia is being used, it will be administered by veterinary personnel trained in anesthetic procedures. Seals receiving general anesthesia will be pre-medicated with atropine (0.02 mg/kg IM). Approximately 10 minutes later, diazepam (5 mg/ml) will be administered IV at a dose rate of 0.25 mg/kg. The seal, relaxed by the diazepam, will then be administered an inhalant anesthetic (Isoflurane at 5%) using a mask. Smaller, more docile seals may be administered inhalant anesthetic without diazepam. Once the seal is anesthetized sufficiently, it will be intubated and maintained on inhalant anesthesia (Isoflurane 1-4%) using an endotracheal tube and a standard field portable gas anesthesia unit with medical oxygen, assisted ventilation capability, a calibrated vaporizer, and a canister for CO2 absorption.

Hair is shaved and collected from the blubber biopsy site for stable isotope analysis or heavy metal analysis and then a sterile scalpel blade is used to make an initial skin incision of ~0.5 cm, just large enough to allow entry of the biopsy tool.

The blubber biopsy is obtained using a sterile, disposable, medical-grade biopsy punch. The blubber biopsy (a 6mm core spanning the full depth of the blubber layer) is taken above the hip and the site is left open to heal (the scalpel incision heals more rapidly than a round hole created by the biopsy punch). Blubber samples are collected to study contaminants and diet, but may also be used for other biochemical analysis. Muscle biopsies are collected for histo-and biochemical analysis to assess nutritional and diving physiology and will only be collected when ADF&G or their collaborators have funding for a specific study that requires the collection of muscle biopsies. The muscle biopsies (approximately 6mm x 1-15mm) are also obtained using standard, sterile, medical biopsy needles (either disposable or multi-use) or sterile disposable biopsy punches. Multi-use biopsy needles will be disinfected after use by flushing the needle and soaking it in a glutaraldehyde solution followed by through flushing with sterile saline prior to re-use. Muscle biopsies will be taken from the hip (longissmus dorsi) during the same biopsy punch insertion as the blubber sample (the blubber biopsy punch is inserted slightly deeper and the muscle is sepeated from the blubber after the "core" is withdrawn). Muscle biopsies may also be taken from the foreflipper (pectoral) regions. The site is left open to heal.

To allow identification of individuals (i.e., previously 'marked') upon subsequent recapture, especially in instances when the flipper tag is lost or is worn so that the number is illegible, a PIT tag (<13mm) is injected intramuscularly. To reduce the potential for tag migration given changing blubber thickness, the PIT tag is injected under the muscle over the distal femur area on the left side of the animal. This procedure takes 2-3 seconds and the PIT tag should remain in place for the life of the seal.

Plastic identification tags (e.g. Rototag 44.45x19x2mm) are attached to both rear flippers. A hole is punched in the webbing of each flipper using a leather punch and the tags are applied with special pliers that hold the tags in place. Tags may remain attached for the lifetime of the seal; however some tag loss invariably occurs. In conjunction with flipper tagging, the small (0.5 cm diameter) skin punch is collected from each flipper and preserved in an ethyl alcohol solution for use in genetics studies.

Mucus membrane swab samples are collected by inserting a sterile swab into the area to be sampled and swabbing the mucus membrane.

Tooth extraction is conducted under general anesthesia. An incisor is withdrawn using dental tooth extraction tools (tooth extraction forceps and tooth elevators). Teeth are used to obtain age estimates. Accurate age estimates are necessary to provide information on age structure of populations to better understand factors driving demography such as age at first reproduction, age-specific pregnancy rates, and age-specific survival. A tooth will be extracted for age estimates from all ages, except pups born within the previous 2-3 months. Recently born pups can be distinguished from older seals and thus constitute known-age seals. Within 2-3 months after weaning it is

difficult to distinguish seals less than 1 year of age from those that are 2-3 years of age because some individuals will have been more successful at foraging than others thus body size, mass, and blubber depth will vary in the first years of life.

Milk samples are collected from lactating females by rolling the seal onto her side and expressing milk from the mammary gland with gentle pinching pressure using a finger and thumb.

Stomach tube insertion is used to subsample stomach content (this procedure is noted at "stomach lavage" in the take table). The length of stomach tube to be inserted will be pre-measured, determining the distance from the seal's mouth to its stomach by holding the tube alongside the seal. The seal will be restrained and a stomach tube speculum or a piece of specially fashioned PVC pipe will be placed into the animal's mouth. The stomach tube will be gently slid down the esophagus into the stomach. Lack of audible inhalations/exhalations from the tube will be used to verify that it entered the esophagus instead of the trachea. Negative pressure will be applied on the stomach tube using a large syringe, and a subsample of stomach contents will be pulled into the tube. Tubes and speculums will be rinsed with water and disinfected by soaking in Nolvasan solution for 20 minutes or more before being used on another animal.

One vibrissa is plucked for diet, stress hormome, or heavy metals analysis.

Morphometrics measurements are taken including standard and curvilinear length, hip girth, maximum girth and axial girth.

Blubber depth is determined non-invasively in the field by measuring blubber depth (Polasek et al. in prep) along the body using a portable ultrasound unit (e.g. Sonosite Titan). Readings are taken by applying water or a water-soluble gel and placing the transducer upon the skin and lightly pressing. Images are captured and measured manually with the ultrasound unit. This process takes 2-5 minutes.

For all of the procedures mentioned above, each sampling site is surgically prepared using alternating 0.2% betadine solution and isopropyl alcohol at least twice or until the site wipes clean, prior to any procedures that break the skin. Tools are either discarded or cold-sterilized after each procedure with a 0.05% chlorhexidine solution.

Once all biological samples are collected, external instruments may be attached. Instruments we may use are as follows:

Satellite tags (back or head mounted): Satellite tags are attached to the dorsal surface using 5-minute epoxy and the procedure takes approximately 10 minutes to complete. Small lightweight tags may be attached to the head of larger animals. The tag will remain attached until molt (~1 year or less) when it will be shed with the fur. The tag is either attached directly to the fur or may be encased in a flotation package with a VHF transmitter to aid in retrieval. SPOT tags transmit location data and haulout statistics through ARGOS satellites and do not require retrieval. SPLASH tags include sensors to measure depth, temperature, light level, and wet/dry periods (to determine hauled out vs. swimming). During the deployment, depth and temperature data are collected, analyzed, summarized, and compressed for transmission through the Argos satellites. Obtaining more detailed individual dive data, also recorded by the instrument, requires that the tag be recovered. Technical specifications: SPOT tag dimensions 71.5x34x24.4mm, mass 78g; SPLASH tag dimensions 106.7x35x38.5mm, mass 145g.

Satellite tags (flipper mounted): Satellite tags are attached to a hind flipper by punching a hole in the flipper webbing and then threading the tag clip through the hole. A small screw is threaded through the end of the clip to secure the tag to the seal. Tag loss will occur over time; rate of tag loss is unknown and new designs are continually developed to reduce tag size and improve attachment mechanism to improve tag retention. Some tearing of the webbing between the digits may occur with tag loss, this is a common injury that harbor seals incur from fighting and/or breeding. We have captured harbor seals with substantial injuries to the flippers that were otherwise healthy and did not seem to be suffering any ill effects or loss of flipper use.This tag transmits location data and haulout statistics through ARGOS satellites and does not require retrieval. Technical specifications: dimensions 58x31.5x18mm, mass 50g.

VHF transmitter (head mounted): VHF transmitters are attached to the seal's head using 5-minute epoxy and the procedure takes approximately 10 minutes to complete. The transmitter will remain attached until molt (~ 1 year or less) when it will be shed with the fur. This instrument emits a VHF frequency and does not collect any data thus does not require retrieval after it is released from the seal. Headmounted VHF transmitters allow researchers to detect the seals while hauled out or while swimming at the surface. The VHF frequency is used to track known seals in order to record locations, directions of travel, etc. Also, land based VHF receivers are used to record harbor seal presence based on VHF frequency detections. Technical specifications: dimensions 35x71x21mm, mass 92g

VHF transmitter (back mounted): VHF transmitters are encased in a flotation package with one or more other instruments (e.g. time-depth recorder, heart rate recorder, archival satellite tag). The package is attached to the dorsal surface using 5-minute epoxy and the procedure takes approximately 10 minutes. The package will remain attached until molt (~ 1 year or less) when it will be shed with the fur. This instrument emits a VHF frequency and does not archive any data. Backmounted VHF transmitters allow researchers or land-based VHF receivers to determine when an unseen seal is hauled out because a backmounted transmitter only emits a steady signal

when the antenna is above water (i.e., when the seal is hauled out). Also, this transmitter is included in a flotation package along with archival instruments to allow for retrieval after it is released. Technical specifications (transmitter only): dimensions 35x60x21x16mm, mass 52g.

Time-depth recorder (TDR): The TDR is encased in a flotation package that also contains one or more other instruments including a VHF transmitter. The package is attached to the dorsal surface using 5-minute epoxy and the procedure takes approximately 10 minutes. The package will remain attached until molt (~1 year or less) when it will be shed with the fur. The TDR measures depth, temperature, and light-level, and also differentiates wet or dry conditions. It must be recovered to retrieve the data. Technical specifications: dimensions 7.3x17.4x17.4mm, mass 35g.

Heart rate temperature recorder (HTR): The HTR is encased in a flotation package that also contains one or more other instruments including a VHF transmitter. The package is attached to the dorsal surface using 5-minute epoxy and the procedure takes approximately 10 minutes. The package will remain attached until molt (~1 year or less) when it will be shed with the fur. The HTR records heart rate data from the heart rate transmitter and must be recovered to access the data. Technical specifications: dimensions 66x45.7x12.7mm, mass 60g.

Heart rate transmitter and electrodes (HRX): The HRX is attached to two electrodes that are fabricated from silver disks mounted in an epoxy base. The HRX and electrodes are attached to the dorsal surface with using 5-minute epoxy and the procedure takes approximately 10 minutes. The package will remain attached until molt (~1 year or less) when it will be shed with the fur. The HRX transmits heart rate data to the HTR and does not need to be recovered. Technical specifications: HRX dimensions 62x31x20mm, mass 47g; electrode dimensions 80x20mm discs.

Flotation package: Flotation packages are custom made from high density foam to house a combination of archival instruments (satellite tags, TDR, HTR, VHF transmitter) that will need to be recovered after release from the seal. Instrument combinations include:

• SPLASH tag and VHF transmitter (dimensions 145x100x37mm, mass ~600g)
• TDR, HTR and VHF transmitter (dimensions 145x100x37mm, mass ~520g)
• TDR and VHF transmitter (dimensions 145x100x37mm, mass ~480g)

The flotation package is attached to the dorsal surface using 5-minute epoxy and the procedure takes approximately 10 minutes. The package will remain attached until molt (~1 year or less) when it will be shed with the fur. Although VHF, TDR, and HTR weight and dimensions remain fairly consistent over time, satellite tag design continually improves thus weight of future packages we deploy is likely to be less than those we previously deployed.

All instruments are aerodynamically shaped to reduce drag. Combined weight of instrumentation attached to a seal will not exceed 3% of the seal's body weight. Instruments attached to pups will be <1% of body weight. The maximum number of tags that an animal would carry would be a combination of a VHF headmount, HRX, and a flotation package containing a TDR, HTR and VHF transmitter.

Administer drugs or chemicals: In addition to the sedative, analgesic ,and reversal drugs mentioned above, we also have emergency and euthanasia drugs available; specifically:

Emergency Drugs: In the event of respiratory arrest, or severely depressed respiration and accompanying cyanosis, Dopram (doxapram hydrochloride; 20mg/ml), a respiratory stimulant will be administered at a dosage of 0.5 - 1.0 mg/kg. The dosage will slowly be given IV or in the event that a vein cannot be clearly accessed, the dose will be administered under the tongue or intratracheal (IT). Epinephrine, a cardiac stimulant, will be administered IT by squirting a dilute mixture of epinephrine (1 ml of 1:1000 Epinephrine diluted in 5-10ml of physiological saline or Lactated Ringers Solution) down the endotracheal tube after the tube has been inserted into the trachea, administering that dosage IV, or as a last resort intracardiac (IC). In the event of bradycardia (slowed heart rate) Atropine, which increases firing of the SA node in the heart, may be administered IM at a dosage of 0.02mg/kg to assure adequate circulation of oxygenated blood.

Euthanasia Drugs: The seal will be anesthetized with inhalant anesthetic or by injection with a sedative drug, or both as sedation may proceed the induction of anesthesia. When the animal is in a surgical plane of anesthesia, a saturated solution of potassium chloride will be administered by intracardiac injection.

Photo-identification: Photo-identification takes place on Tugidak Island with photos being shot from a cliff 20-40 meters behind and above the seals. Southwest beach is photographed approximately 5 days a week; Middle beach approximately 2 days a week. Seven hours are spent per day photographing systematically across a beach until the entire beach is surveyed, or daylight diminishes. A seal may be photographed multiple times in a day if it hauls out in different spots in a day as we photograph across the beach. Multiple photographs (usually two to five) of each seal are taken if the ventrum is showing. All views (except dorsum views only) of the seals are

photographed if it is tagged, significantly scarred, or includes moms/pups and pregnant seals during pupping season. If there is a tagged/scarred seal 5 to 20 minutes will be spent trying to get good photographs. Researchers work carefully and from a distance to avoid disturbing the seals. A disturbance would cause the seals to move from land into the water.

Vessel survey: Radio-tracking surveys are generally conducted from May through September but may occur year-round. Surveys are typically conducted from a 6.5m Boston Whaler, but may also occur from larger vessels chartered for other purposes (e.g. captures, equipment installation). These surveys do not require approaching the seals, but incidental disturbances may occur.

Approaches: Periodically from May-early August seals equipped with bio-logging equipment are detected within the survey area and slowly (1.5-8 km/hr) approached by a research vessel (e.g., 6.5m Boston Whaler) while researchers record the timing of behaviors such as head lifting, repositioning and entering the water. These data are used to estimate energetic costs of vessel disturbance to harbor seals. Approaches take 1-10 minutes to conduct, depending on starting distance and water/ice conditions, and the vessel backs off as soon as the target animal enters the water. No planned approaches will be conducted from June 1 to June 25 so that mother/pup pairs are not disturbed. Peak pupping occurs approximately the first week in June and the lactation period lasts for 24 days. Planned approaches will resume on June 26th and will continue until molt when the hardware falls off or until sufficient known disturbances have been observed. Up to 50 seals may be approached; a single individual will be approached no more than 5 times per year with an interim of ~4 days between approaches.

Import/export samples: In order to maximize the information from the research that will be conducted, some specimens will need to be exported. We currently collaborate with researchers in Canada and anticipate that we may work with researchers and laboratories from other countries as well. Therefore, we are requesting authority to export samples on a worldwide basis as the need arises. Some specimens exported will be destroyed in the course of analysis and will not need to be returned. Prey remains from scat samples are returned after analysis.

Scat collections: Scats will be collected from terrestrial and ice haulout sites. To collect the scats, known haulout sites will be approached either on foot, by small boat (e.g., Boston whalers, inflatable rafts, or kayaks) or by floatplane (landing ¼ mile offshore and slowly taxiing toward the haulout). The number of seals hauled out will be counted prior to approach. Once a count is obtained, the haulout site will be approached very slowly so that the seals can see the approaching person/boat/plane and slowly move into the water. With a slow approach, we allow ample time for the seals to move into the water at their own pace; once in the water, we often see many animals milling around near the haulout, observing us. Any seal that moves into the water in response to the approaching person, boat, or plane, is considered harassed. We will increase our chance of finding scats (and thus decrease failed attempts which decreases the number of disturbances) by limiting our collections to haulout substrates such as sand, gravel or rocky beaches, or icebergs. Scat is easily found and collected on these substrates compared to haulout substrates dominated by mussel-kelp beds. Scats will not be collected during the pupping or nursing period (mid May through July; varies depending on region) unless seals are disturbed from their haulout during other permitted activities (i.e., tagging).

Scat collections will be made seasonally in winter (November–March), spring (April–May), and late summer/fall (August–October).

Supplemental Information

Status of Species: The 2009 NMFS stock assessment report (Allen and Angliss 2010) provided the following estimates of harbor seal stock abundance: statewide 180,017; Southeast Alaska 112,391; Gulf of Alaska 45,975; and Bering Sea 21,651.

Surveys conducted in the Ketchikan area indicate that the number of harbor seals in that region has been increasing since the mid-1980s, whereas the number in the Sitka region has remained stable (Small et al. 2003). Counts from these regions indicate that the Southeast stock is increasing or stable, however a severe decline has been reported in Glacier Bay during the 1990s (Mathews and Pendleton 2006) that has persisted through surveys conducted in 2007-2008 (Womble et al 2010).

The overall trend within the Gulf of Alaska stock is unclear. Although the long-term trend (1990-2006) in Prince William Sound indicates that seals are declining at an average rate of 2%/yr (ADF&G unpublished), recent data indicate the population began to slowly increase after 2001 with a 5-yr trend from 2002-06 of a 3.3%/yr increase (ADF&G unpublished).

Pitcher (1991) documented an 86% decline in mean molt-period counts on Tugidak Island (near Kodiak) during 1976-1990. Surveys conducted during 1993-2001 indicate that the number of seals in the Kodiak region increased (Small et al. 2003).

A comparison between counts in the Aleutian Islands obtained from 1977-1982 and 1999 indicates a substantial (67%) decline (Small et al. 2008). For the Bering Sea stock, counts along the north side of the Alaska Peninsula and at Nanvak Bay appear to be stable or increasing (Jemison et al. 2006, Small et al. 2003, ADF&G unpublished).

None of the three Alaska harbor seal stocks are currently classified as strategic; however, harbor seals are listed as a species of concern by the State of Alaska. Similarly, the Marine Mammal Commission listed Alaskan harbor seals as a species of concern following the decline of harbor seals documented at Tugidak Island.

Lethal Take:

Seals may drown in the course of being captured or die during processing as a result of drug complications. From 1992-2010 ADF&G captured 1,808 seals with a total of 9 mortalities (0.5%). Events can occur that may result in multiple mortalities during a single year. Therefore, we request a maximum unintentional lethal takes of 4/yr not to exceed 10/duration of the permit.
As per the ACUC requirements for this project, any wildlife research must have a plan for humane euthanasia, conducted in a manner approved by the Guidelines for the Euthanasia of Nondomestic Animals. The criteria used to determine if euthanasia is warranted is discussed in the "Euthanasia" attachment. We request a maximum intentional lethal takes of 2/year not to exceed 5/duration of the permit.

Anticipated Effects on Animals:

Anticipated effects of each activity alone:

Capture- once entangled in the net, most animals struggle to escape. A small number of seals receive small cuts and abrasions from the net. Seals entangled close together in the same net may bite one another. Seals likely experience stress and expend energy during captures. Seals are removed from the net as soon as possible.

Physical restraint- seals are restrained twice, first to remove them from the net and secondly to undergo the sampling procedures. Animals likely experience stress during restraint. Although, when restrained properly, most seals do not struggle excessively during net disentanglement, and minimally during the pre-sedation portion of sampling.

Weighing- seals contained within a hoop net or brailer bag are weighed as they are hoisted onto the main vessel with a crane. Alternatively, seals may be weighed by being suspended inside a hoop net or brailer bag from a board or oar held aloft by 2 crew members. Seals likely experience stress while being weighed.

Blood draw- In order to collect uncontaminated blood samples, blood is drawn before the sedative is injected. Seals likely experience some discomfort during the needle insertion.

Drug injection- sedatives are injected through the needle that the blood was drawn from, prior to its removal from the venous space. In most cases, seals visibly relax (decrease muscle tension) almost immediately upon sedative administration, and it is not uncommon for seals to fall asleep during the sampling procedure. An analgesic is injected to decrease discomfort over the following hours to days. After the sample collection is completed, animals are moved to a quiet location to recover from the sedative, or a sedative reversal agent is administered intramuscularly (IM). During IM injections, if the animal moves or the blubber layer is thicker than expected, the analgesic or sedative reversal agent could be injected subcutaneously (SQ). The drug would still be absorbed by the animal, it would just be absorbed more slowly. An additional needle insertion is not necessary to administer the sedative, and the analgesic and reversal agent are administered while the animal is sedated, so additional stress is probably not endured for drug administration. Diazepam has been shown to block memory, so the sample collection procedures are likely not incorporated into the seals permanent memory.

Hair collection- because harbor seal hair is not insulative, no negative effects are anticipated.

Blubber and muscle biopsy- The biopsy site is not sutured shut because closing the wound does not allow the area to drain, thus increasing the likelihood of infection. When the animal enters the cold water, the skin and subdural tissues contract and the biopsy site closes. We have recaptured seals days after a biopsy has occurred and the wound was closed and beginning to heal over. There may be residual soreness that persists for days at the biopsy site, but the trauma from the biopsy is far less than trauma observed from conspecific fighting or bite wounds from seal predators that are commonly observed on seals we capture.

PIT tag insertion- There is likely residual soreness that persists for days at the PIT tag insertion site, but the trauma from the tag insertion is far less than trauma observed from conspecific fighting or bite wounds from seal predators that are commonly observed on seals we capture.

Plastic Identification tags- a small hole is punched into the flipper to allow the insertion of the ID tags. Some tags may tear out of the flippers over time. We have observed large tears in flippers of seals that we have not previously captured, likely incurred during fights with conspecifics or evasion of predators. These tears are often considerably larger than the small holes punched for flipper tags and the length of a tear should a flipper tag be torn out. The large, naturally incurred injuries are generally completely healed and do not appear to impact flipper function (based on observations of seals swimming away following processing).

Mucus swab- no negative effects are anticipated.

Vibrissa collection- A vibrissa is pulled out of the muzzle. Some soreness may occur.

Ultrasound- no negative effects are anticipated.

Milk expression- no negative effects are anticipated.

Stomach tube-aspiration or trauma are possible if a tube is improperly passed into the stomach.

Attachment of external instruments-increased drag when swimming and discomfort from hair being pulled are potential negative effects associated with external instrument attachment.

General anesthesia- Use of general anesthesia has a mortality risk.

Tooth extraction- results in trauma and likely results in lingering pain, potentially for a few days.

Cumulative effect of capture and sample collection: Stress is poorly understood in free ranging animals, and individuals respond to stress differently based on age, sex, season, and prior exposure. When an animal encounters a stressor, a cascade of hormones is released. This causes the fight or flight response and the mobilization of body stores to increase the availability of circulating glucose to fuel fight or flight. So, during capture events, harbor seals will likely incur an energetic cost. This is an adaptive response to a stressor and is used for survival when confronted by predators, or other stressors in the environment (e.g., tour boats). Our captures are a single event causing stress for a finite amount of time, probably not different from an occasion when harbor seals are presented with the presence of a predator. Once captured, seals are held for a limited amount of time, administered drugs to decrease pain and block memory and seals are rarely recaptured. Of the total captures we have recorded from 1994, only 51 were recaptured, representing a 3% recapture rate, and only two animals were recaptured and retained for processing in the same year. The short-term, acute stress associated with our captures does not persist for a long period of time and is rarely repeated. Therefore, our captures do not fit the definition of chronic stress, where a stressor is encountered repeatedly for an extended period of time, which can negatively impact animal health.

Anticipated effects on the population: It is unlikely that there are any effects at the population stock level. ADF&G has historically conducted captures in multiple locations for many years with no apparent effect on abundance; seals continue to use haulouts where captures were conducted over the years and population trends in some areas (e.g., Tugidak and PWS) have changed from declining to increasing during the time in which captures occurred in the area.

Mortalities: During the previous five years for Permit #358-1757 a total of 559 seals were captured and no mortalities occurred.

Conspecifics or non-target species: Harbor porpoises, Steller sea lions, sea otters, various waterfowl, humpback whales, and/or orcas may be encountered en route to or from our study sites. We are careful to moderate our vessel speed and path to avoid marine mammals per NMFS guidelines. When conducting captures, we take precautions to assure that we are setting nets on our target species, including cessation of net setting or opening/removing the net to allow their escape if non-target species are seen in the capture area. We rarely encounter Steller sea lions, sea otters and whales at harbor seal haul outs (i.e., capture sites). However, harbor porpoises are commonly found in the same areas where we conduct captures. Unlike Steller sea lions and sea otters, harbor porpoises are generally encountered in very small groups, they spend a large amount of time submerged and are difficult to detect. Therefore, it is possible that a harbor porpoise would be entangled in our nets before we were aware of its presence in the area and had the opportunity to move the net. In the past 9 years of captures, we have only had one harbor porpoise entangled in our net, which was released unharmed, and have not entangled any other non-target species.

Scats are collected on substrate not generally used by other species, however we avoid scat collection during times when our approach to the terrain might involve disturbance to non target species; e.g., if scat collection occurs at a site that seasonally hosts nesting birds, if approach to scat collection site would disturb molting flocks of waterfowl.

Non-target species may be temporarily disturbed during capture attempts or radio tracking, although harbor seals generally do not haul out with non-target species, thus effects on non-target species are minimal. Effects on conspecifics are mixed. At some study sites, harbor seals that we captured the previous day (those visually identifiable via color-coded external transmitters) were observed closely approaching our capture skiffs and nets the following day, while in other areas we have observed temporary hyper-vigilance to vessel presence in some seals, with particular sensitivity to vessels similar to ours, following a capture event. This phenomenon was noted when a subset of researchers remained in the field to radio track after the capture team left the area. If the skiff used for net setting kept moving, well offshore (>200m), while passing a seal haulout, seals did not react. However, if the skiff stopped anywhere within sight, or more likely within hearing range of the haulout, even after having passed the haulout, a few seals would enter the water when the skiff stopped. After a day or two of seeing our skiff in the vicinity of haulouts and not having our skiff approach the haulouts, the seals stopped reacting to the nearby passage (>100m) of our skiff. On subsequent radio-tracking trips, seals also did not show any reaction to our skiff if we remained offshore (>100m). Short-term (1-2 days) following captures, seals may expend more energy by being

hyper-reactive to vessel presence, if vessels stop within hearing distance of haulouts, however most vessels in our study areas are in transit and do not stop. The post-capture effect for stopping vessels diminishes rapidly and seals resume their normal behavior toward vessel presence. To avoid harassment following a capture trip, we generally do not remain in the area unless critical data must be obtained immediately post capture. We also generally do not return to the same area to conduct additional captures for a minimum of 2-3 months, and no more than four capture trips will occur in a single area within the same year. Thus, if hyper-vigilance for boats occurs for some seals, the effect will be brief.

Measures to Minimize Effects to Listed Species:

Measures to mitigate negative effects for each activity:

Capture- nets are monitored at all times and checked often to avoid accidental drowning. Seals are removed from the net as soon as possible. If there is more than one seal captured, every effort is made to prevent the animals from biting each other (e.g., placing bulky objects between seals and extracting the least entangled seal quickly).

Physical restraint- individuals that are performing restraint are first trained by an experienced individual. While restraining the animal, respirations are monitored continuously and no pressure is placed on top of the animal's thoracic area to avoid restriction of the heart and lungs.

Weighing- seals are weighed as quickly as possible, avoiding extended time elevated above the deck. Care is taken to ensure no claws or teeth are caught in the hoop net webbing prior to being hoisted. Large animals that may exceed the weight bearing abilities of the hoop net are first placed into brailer bags before being hoisted.

Blood draw- prior to needle insertion, the area is scrubbed with betadine and alcohol. Only sterile needles are used. If the venous cavity is not reached after 3 needle insertions, the blood draw is aborted and the animal then receives a shortened work up without blood being drawn or sedatives being injected.

Drug injection- sedatives are injected through the needle that the blood was drawn from prior to its removal from the venous space. The analgesic and reversal agent are administered while the animal is sedated, so additional stress is probably not endured for drug administration. Diazepam has been shown to block memory, so the sample collection procedures are likely not incorporated into the seals permanent memory. After each needle is removed, pressure is applied to the area to decrease the likelihood of external bleeding or subdermal hemotoma formation.

Hair collection- in most cases the animal is sedated when the hair is collected. Because harbor seal hair is not insulative, no negative effects are anticipated.

Blubber and muscle biopsy- the animal is sedated when the biopsy occurs. The area is shaved and scrubbed with betadine and alcohol. The skin is cut with a sterile scalpel blade, thus decreasing the amount of trauma to the dermis. After the biopsy is taken, pressure is applied to the area to decrease the likelihood of external bleeding or subdermal hemotoma formation. An analgesic is administered to reduce discomfort associated with this procedure.

PIT tag insertion- in most cases the animal is sedated when the PIT tag insertion occurs. The area is scrubbed with betadine and alcohol. After the PIT tag is inserted, pressure is applied to the area to decrease the likelihood of external bleeding or subdermal hemotoma formation. An analgesic is administered to reduce discomfort associated with this procedure.

Plastic Identification tags –in most cases the animal is sedated when the ID tags are affixed. The area is scrubbed with betadine and alcohol and a hole is punched into the flipper webbing to decrease the trauma associated with tag attachment. After the ID tag is affixed, the sharp point is removed to decrease the likelihood of abrasion. An analgesic is administered to reduce discomfort associated with this procedure.

Mucus swab- in most cases the animal is sedated when the mucus swab occurs. No negative effects are anticipated.

Vibrissa collection- in most cases the animal is sedated when the vibrissa is pulled. An analgesic is administered to reduce discomfort associated with this procedure.

Ultrasound- in most cases the animal is sedated when the ultrasound is conducted. No negative effects are anticipated.

Milk expression- No negative effects are anticipated.

Stomach tube insertion-only trained individuals will insert stomach tubes. Care will be taken to avoid aspiration injury by listening to the tube and only proceeding if there is an absence of inhalation/exhalation

noise associated with being in the trachea instead of the esophagus. When the stomach tube is removed the end is held closed to create a vacuum, retaining any remaining contents to prevent them from being expelled and aspirated. Stomach tubes will be rinsed and soaked in a disinfecting Nolvassan solution between uses to decrease the likelihood of disease transmission.

Attachment of external instruments- . There is no way to completely mitigate drag associated with instrument attachment; however, we use the most aerodynamically designed and smallest possible instruments to reduce drag and minimize the attachment footprint. Generally these instruments are attached to pelage and only remain in place until the following molt (~1yr or less). Multiple studies have assessed the effects of handling and tagging of pinnipeds of all ages and found no adverse effects (McCafferty et al. 2007, McMahon et al. 2005, 2008, Baker and Johanos 2002). Tagging of harbor seals as young as 2 days old has occurred (Greaves et al. 2005) with no report of adverse effects. We do not tags seals at that early lifestage and pups tagged near weaning are tagged with instruments that weigh proportionately less than the tags used in the Greaves et al. 2005 study.

General anesthesia-The safest possible drugs with the shortest effect are used and are administered by personnel specifically trained in anesthesia procedures. Use of general anesthesia at deeper levels results in complete analgesia (eliminating stress from painful stimuli). General anesthesia also prevents movement and any perception of sampling procedures by the anesthetized seal, thus reducing stress and potential for injury to the invidual if movement occurred at an inopportune time.

Tooth extraction- Our earlier research determined that the smallest, least-used tooth – an incisor adjacent to the canine – can be extracted and provides accurate age estimates. Because that tooth is next to a much larger canine tooth, the wound resulting from tooth extraction likely has minimal contact with prey that are captured or with other surfaces while healing. The high degree of vascularization results in rapid healing of injuries of the gum and constant flushing with salt water prevents infection while the wound from extraction of a small incisor heals.

We attempt to conduct all captures in areas where the chance of disturbing other wildlife is minimal. During terrestrial captures, nets are set only when harbor seals are confirmed to be hauled out on the site being approached. In glacial ice, generally harbor seals are the only species in the area, although occasionally harbor porpoises and, rarely, a Steller sea lion are seen. When a net is in the water, if a non-target species is observed within the net, the net is opened to allow the non-target animal(s) to escape even if it results in all harbor seals also escaping. If a non-target species is accidentally captured, the animal is released as quickly as possible. The capture techniques that we use are the most efficient and effective for capturing harbor seals and our low mortality rate attests to the safety of the procedures that we use.

We use sedatives when handling seals to minimize their stress during the sampling procedures, provided that the sedatives do not compromise the well being of the individual. We also administer analgesics to minimize the pain experienced by the seals during processing and afterwards. For more intensive procedures we use general anesthesia administered by trained professionals. If we do not use a sedative, the procedures that are considered surgery under our ACUC (e.g., blubber and muscle biopsy) are not conducted. The equipment that we use to obtain biological samples, and the sedatives, analgesics, and/or anesthetics that we use are selected based upon current veterinary procedures, as verified by our veterinary collaborators and our ACUC permitting process.

Processing of seals is done as efficiently as possible by experienced researchers. Specific age and sex groups that are prioritized to process and release first are those cohorts more likely to experience stress or lost opportunities (e.g., nursing or breeding) compared to other groups. For example, during the breeding season, single, dependent pups are processed and released first, and reproductive-aged females are processed before juveniles and sub-adults. Seals are released as soon as they are alert and exhibit behavioral traits similar to those observed for the individual prior to sedation.

All of our research is conducted in collaboration with other agencies studying harbor seals in Alaska. Collaborators generally accompany us on each capture trip, samples are shared with multiple collaborators (including agencies and individuals not participating in specific capture trips). We often deploy instruments during our captures that enable data collection for other agencies (e.g., NMFS), and data from aerial surveys to determine abundance and population trends are shared between NMFS and ADF&G.

Resources Needed to Accomplish Objectives:

We have a large number of co-investigators because we need a pool of people from which to select at any given time and our captures require many trained and experienced individuals. At times we may be widely dispersed in a single location or have several activities occurring at once (field camps, aerial surveys, captures, resight trips, etc.)

Experienced researchers following established procedures will conduct or closely supervise the capture, physiological measurements, sampling, and instrumentation of harbor seals. The proposed methods have been used previously by ADF&G and NMFS for studies of seals in Alaska and elsewhere.

Additional resources are listed in the attachment, "Resources needed to accomplish objectives".

Disposition of Tissues: Samples collected may include blood, hair, whisker, skin, blubber, muscle, mucosal swab, milk, scat and tooth. Most samples are destroyed in the analysis process. Tooth samples have a thin section removed and mounted on a slide during processing. Fatty Acid Methyl Ester (FAME) retained after blubber fatty acid analysis are returned to ADF&G and archived for further analysis (e.g., hormones). Post-processing, the teeth and slides are returned to ADF&G for storage, as are hard-parts extracted from scat for prey identification. Blood samples that are archived for future research will be stored in a -80 degree freezer located at the ADF&G office in Anchorage or at the Alaska SeaLife Center in Seward. Hair and whisker samples that are archived will be stored in the Douglas ADF&G office.

Public Availability of Product/Publications: Research may be presented at scientific conferences (e.g., Alaska Marine Science Symposium and Society of Marine Mammalogy Conference) and public meetings, summarized in federal funding and permitting reports and published in professional journals. A list of published journal articles of research conducted by or in collaboration with ADF&G is available to the public on the ADF&G website (http://www.adfg.alaska.gov/index.cfm?adfg=marinemammalprogram.publications).

Location/Take Information

Location

Research Area: Pacific Ocean **State:** AK
Location Description:

Take Information

Line	Ver	Species	Listing Unit/Stock	Production /Origin	Life Stage	Sex	Expected Take	Takes Per Animal	Take Action	Observe /Collect Method	Procedure	Transport Record	Begin Date	End Date
1		Porpoise, harbor	Range-wide	Wild	All	Male and Female	5	2	Capture/Handle/Release	Net	Incidental harassment	N/A	1/1/2012	12/31/2016
		Details: Unintentional capture of harbor porpoise during capture activities directed at harbor seals												
2	A	Seal, harbor	Range-wide	Wild	All	Male and Female	350	2	Capture/Handle/Release	Net, other	Administer drug, IM ; Administer drug, IV; Administer drug, subcutaneous; Anesthesia, gas w/cone or mask; Anesthesia, gas w/intubation; Anesthesia, injectable sedative; Instrument, internal (e.g., PIT); Mark, flipper tag; Measure (standard morphometrics); Photogrammetry; Restrain, board; Restrain, hand; Restrain, net; Restrain, other; Sample, blood ; Sample, blubber biopsy; Sample, clip hair; Sample, milk (lactating females); Sample, muscle biopsy; Sample, skin biopsy; Sample, stomach lavage; Sample, swab all mucus membranes; Sample, tooth extraction; Sample, vibrissae (pull); Ultrasound; Weigh	N/A	6/1/2012	12/31/2016
		Details: Intentional capture of harbor seals using a drift monofilament net, multi-filament seine net, hoop net or dip net. Most captures are conducted using a monofilament net (used in glacial habitat) or multi-filament seine net (used in terrestrial habitat).												

3		Seal, harbor	Range-wide	Wild	All	Male and Female	2000	50	Import/export/receive only	Other	Import/export/receive, parts	N/A	1/1/2012	12/31/2016
		Details: export samples to Canada for analysis												
4		Seal, harbor	Range-wide	Wild	All	Male and Female	1750	50	Unknown	Other	Salvage (carcass, tissue, parts)	N/A	1/1/2012	12/31/2016
		Details: Collect samples from subsistence harvested animals and other mortalities												
5		Seal, harbor	Range-wide	Wild	All	Male and Female	180000	12	Harass	Survey, aerial	Incidental disturbance	N/A	1/1/2012	12/31/2016
		Details: Incidental disturbance during aerial surveys												
6		Seal, harbor	Range-wide	Wild	All	Male and Female	4	1	Unintentional mortality	Other	Unintentional mortality	N/A	1/1/2012	12/31/2016
		Details: Unintentional research related mortality of animals during capture or sampling activities. We request a take of 4/yr not to exceed 10 for the duration of the permit.												
7		Seal, harbor	Range-wide	Wild	All	Male and Female	7000	36	Harass	Other	Incidental disturbance	N/A	1/1/2012	12/31/2016
		Details: incidental disturbance during capture activities												
8		Seal, harbor	Range-wide	Wild	All	Male and Female	10000	120	Harass	Survey, ground	Collect, scat; Incidental disturbance	N/A	1/1/2012	12/31/2016
		Details: incidental disturbance during photo-id, count surveys, behavioral observations and scat collection												
9		Seal, harbor	Range-wide	Wild	All	Male and Female	50	5	Harass	Survey, vessel	Observations, behavioral	N/A	1/1/2012	12/31/2016
		Details: Intentional disturbance of animals equipped with bio-logging equipment												

10		Seal, harbor	Range-wide	Wild	All	Male and Female	4000	36	Harass	Survey, vessel	Incidental disturbance	N/A	1/1/2012	12/31/2016
	Details: Unintentional disturbance of animals in conjunction with radio-tracking or in conjuction with intentional disturbance of animals equipped with bio-logging equipment													
11		Porpoise, harbor	Range-wide	Wild	All	Male and Female	3	1	Unintentional mortality	Other	Unintentional mortality	N/A	1/1/2012	12/31/2016
	Details: Unintentional mortality of harbor porpoise during harbor seal capture activities													
12		Seal, harbor	Range-wide	Wild	All	Male and Female	2	1	Intentional (Directed) Mortality	Other	Intentional (directed) mortality	N/A	1/1/2012	12/31/2016
	Details: Humanely euthanize animals seriously injured during research. We request a take of 2/yr not to exceed 5 for the duration of the permit.													
13		Seal, harbor	Range-wide	Wild	All	Male and Female	4	1	Capture/Handle/Release	Net, other	Other	N/A	1/1/2012	12/31/2016
	Details: administer emergency drugs intratracheal or intracardiac if other drug delivery methods are unsuccessful.													
14		Seal, harbor	Range-wide	Wild	All	Male and Female	150	2	Capture/Handle/Release	Net, other	Instrument, external (e.g., VHF, SLTDR)	N/A	1/1/2012	12/31/2016
	Details: A subset of the seals captured and processed (line 2, above) may have external transmitters attached.													

Location

Research Area: Pacific Ocean **State:** AK
Location Description: Alaska SeaLife Center, Seward, AK

Take Information

Line	Ver	Species	Listing Unit/Stock	Production /Origin	Life Stage	Sex	Expected Take	Takes Per Animal	Take Action	Observe /Collect Method	Procedure	Transport Record	Begin Date	End Date

1	A	Seal, harbor	Range-wide	Captive	All	Male and Female	9	75	Captive animals (research, enhancement, public display)	Captive	Administer drug, IV; Anesthesia, injectable sedative; Mark, dye or paint; Photogrammetry; Restrain, hand	N/A	6/1/2012	12/31/2016
	Details: captive seals at ASLC, includes current inventory and animals added during the project													
2	A	Seal, harbor	Range-wide	Rehabilitation Facility	All	Male and Female	15	75	Captive animals (research, enhancement, public display)	Captive	Administer drug, IV; Anesthesia, injectable sedative; Mark, dye or paint; Photogrammetry; Restrain, hand	N/A	6/1/2012	12/31/2016
	Details: transient rehab animals held at ASLC													
5	A	Seal, harbor	Range-wide	Captive	All	Male and Female	3	1	Unintentional mortality	Captive	Other	N/A	6/1/2012	12/31/2016
	Details: research related (unintentional) mortality													
6	A	Seal, harbor	Range-wide	Rehabilitation Facility	All	Male and Female	5	1	Unintentional mortality	Captive	Other	N/A	6/1/2012	12/31/2016
	Details: research related (unintentional) mortality													

NEPA Checklist

1) If your activities will involve equipment (e.g., scientific instruments) or techniques that are new, untested,or otherwise have unknown or uncertain impacts on the biological or physical environment , please discuss the degree to which they are likely to be adopted by others for similar activities or applied more broadly.

The research we are proposing is a continuation of the work ADF&G has been conducting for over a decade, and we believe there are no unique or unknown risks. The techniques we will use are standard and not controversial. Similar techniques are used for studying pinniped biology worldwide.

2) If your activities involve collecting, handling, or transporting potentially infectious agents or pathogens (e.g., biological specimens such as live animals or blood), or using or transporting hazardous substances (e.g., toxic chemicals), provide a description of the protocols you will use to ensure public health and human safety are not adversely affected, such as by spread of zoonotic diseases or contamination of food or water supplies.

The proposed research involves the handling of live animals and the collection of blood, tissues, and scat. All personnel handling live animals have been trained in safe animal handling procedures and wear gloves and other protective clothing when capturing and/or restraining animals. Latex or nitrile gloves are worn during the collection and handling of blood, tissues, and scat. All samples are stored in sealed containers (e.g. whirlpak bags, cryovials) and are only handled by researchers.

No aspect of this research should affect the public health or safety provided seals are not subsistence-harvested within 45 days of their capture and their products consumed. There are no established drug withdrawal times for marine mammals, thus the FDA recommendation is 45 days.

3) Describe the physical characteristics of your project location, including whether you will be working in or near unique geographic areas such as state or National Marine Sanctuaries, Marine Protected Areas, Parks or Wilderness Areas, Wildlife Refuges, Wild and Scenic Rivers, designated Critical Habitat for endangered or threatened species, Essential Fish Habitat, etc. Discuss how your activities could impact the physical environment, such as by direct alteration of substrate during use of bottom trawls, setting nets, anchoring vessels or buoys, erecting blinds or other structures, or ingress and egress of researchers, and measures you will

take to minimize these impacts.

Photo-identification and scat collection will take place on Tugidak Island Critical Habitat Area that features a large shallow lagoon and barrier spit complex and low lying tundra relief. Ingress and egress of researchers is by aircraft at a designated allowable location (Pick-up Point) and vehicle access is limited to 4-wheelers that are only allowed on non-vegetated portions of the beach. Passage from Pick-up Point to the field camp involves disturbing seals, however scat collection occurs at that time, facilitating a long-term study of diet.

Project locations have not been determined for future capture activities, but will likely involve previously used sites as well as new areas. Past similar research has had little to no impact on the physical environment. Remote telemetry stations may be installed in areas where captures take place, however care is taken to install them in a manner that causes minimal vegetation disturbance. The appropriate permits are obtained prior to equipment deployment, and stations are camouflaged to minimize the visual disturbance they might cause in a high human traffic environment.

4) Briefly describe important scientific, cultural, or historic resources (e.g., archeological resources, animals used for subsistence, sites listed in or eligible for listing in the National Register of Historic Places) in your project area and discuss measures you will take to ensure your work does not cause loss or destruction of such resources. If your activity will target marine mammals in Alaska or Washington, discuss measures you will take to ensure your project does not adversely affect the availability (e.g., distribution, abundance) or suitability (e.g., food safety) of these animals for subsistence uses.

The proposed research targets harbor seals in Alaska. All proposed research is conducted in a manner to minimize disturbance and will not adversely affect the long-term distribution or abundance of harbor seals in Alaska. If research is conducted in an area that is used for subsistence hunting, we will work with local Alaska Native organizations to ensure that research and hunting do not negatively impact each other. Besides communication with local organizations, ADF&G researchers annually attend meetings with all researchers conducting harbor seal research in Alaska to discuss ongoing research; representatives from the Alaska Native Harbor Seal Commission (ANHSC) are included in those meetings.

Hunters are encouraged by ANHSC (verbally, website and newsletter) to contact ADF&G or ANHSC, and provide the flipper tag numbers should they take a seal that has ADF&G flipper tags to assure that all parts of the seal are safe to consume.

5) Discuss whether your project involves activities known or suspected of introducing or spreading invasive species, intentionally or not, (e.g., transporting animals or tissues, discharging ballast water, use of equipment at multiple sites). Describe measures you would take to prevent the possible introduction or spread of non-indigenous or invasive species, including plants, animals, microbes, or other biological agents.

Capture equipment (e.g. capture nets, hoop nets) may be used at multiple sites within a year. After each capture trip the equipment is washed, cleaned of all debris, sanitized and allowed to fully dry in a storage facility. Chartered vessels used for captures are generally obtained locally, or from within distances and pathways normally traversed by numerous vessels other than our research charters.

Project Contacts

Responsible Party: Robert Small
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Juneau, AK 99811-5526
Phone: (907)465-6167
Email: Bob.Small@alaska.gov

Primary Contact: Christine Schmale

Principal Investigator: Gail Blundell

Other Personnel:

Name	Role(s)
James Bailey	Veterinarian
Jonathan Barton	Co-Investigator
Rachel Dziuba	Veterinarian

Tom Gage	Co-Investigator
Sue E. Goodglick	Co-Investigator
Kelly Hastings	Co-Investigator
Lauri Jemison	Co-Investigator
Shawna Karpovich	Co-Investigator
Grey Pendleton	Co-Investigator
Lori Polasek	Co-Investigator
Jill Prewitt	Co-Investigator
Christine Schmale	Co-Investigator
Robert Small	Co-Investigator
Justin Smith	Co-Investigator
Jamie Womble	Co-Investigator
Kate M Wynne	Co-Investigator

Attachments

Application Archive - P16094T14Issued.pdf (Added Oct 24, 2011)
Contact - Christine Schmale: C11704T5Christine Schmale CV.doc (Added Jul 11, 2008)
Contact - Gail Blundell: C8195T5Blundell_ NMFS permit_CVITAE_10.docx (Added Oct 26, 2010)
Contact - Gail Blundell: C8195T5Blundell_ NMFS permit_CVITAE_10v2.docx (Added Feb 7, 2011)
Contact - Gail Blundell: C8195T5GAIL Blundell CV.doc (Added Jul 11, 2008)
Contact - Grey Pendleton: C13060T5Pendleton CV.doc (Added May 7, 2009)
Contact - Grey Pendleton: C13060T5Pendleton_CV permit 29oct10.docx (Added Nov 2, 2010)
Contact - James Bailey: C8683T5James Edward Bailey.doc (Added Feb 1, 2011)
Contact - Jamie Womble: C14713T5JamieWomble_ADFGPermitApp_2010.docx (Added Nov 2, 2010)
Contact - Jill Prewitt: C9937T517263_Prewitt CV.docx (Added Jun 18, 2012)
Contact - Jill Prewitt: C9937T5Jill Prewitt CV 2010.docx (Added Nov 5, 2010)
Contact - Jill Prewitt: C9937T5Jill Prewitt CV.DOC (Added Oct 13, 2009)

Contact - Jonathan Barton: C14714T5Barton CV 2010.docx (Added Feb 1, 2011)
Contact - Justin Smith: C14715T5justin smith HS permit CV.docx (Added Nov 2, 2010)
Contact - Kate M Wynne: C13441T5CV2009full.SSLPermit.doc (Added Sep 17, 2009)
Contact - Kate M Wynne: C13441T5Wynne CVHSpermit2010.doc (Added Oct 26, 2010)
Contact - Kate M Wynne: C6580T5KWynne_CV.doc (Added Feb 11, 2009)
Contact - Kelly Hastings: C9831T5Hastings CURRICULUM VITAE.doc (Added May 1, 2009)
Contact - Kelly Hastings: C9831T5Hastings CV June2010.doc (Added Oct 26, 2010)
Contact - Lauri Jemison: C6569T5CV_LAJ_2012.pdf (Added Jun 6, 2012)
Contact - Lauri Jemison: C6569T5Jemison CURRICULUM VITAE.doc (Added May 1, 2009)
Contact - Lori Polasek: C13277T5Polasek CV 10-26-10 Abbreviated.pdf (Added Oct 26, 2010)
Contact - Lori Polasek: C13277T5Polasek CV 5-15-09.pdf (Added Jul 20, 2009)
Contact - Lori Polasek: C8751T514324 Polasek CV.doc (Added Apr 30, 2009)
Contact - Rachel Dziuba: C14937T5Dziuba CV 2010.doc (Added Feb 1, 2011)
Contact - Robert Small: C6568T5Bob Small CV.doc (Added Jul 11, 2008)
Contact - Robert Small: C6568T5RJS CV.pdf (Added Nov 2, 2010)
Contact - Shawna Karpovich: C11702T5Shawna Karpovich CV.doc (Added Jul 11, 2008)
Contact - Sue E. Goodglick: C13063T5Goodglick CV HS 2010.docx (Added Nov 2, 2010)
Contact - Sue E. Goodglick: C13063T5Goodglick CV.pdf (Added May 8, 2009)
Contact - Tom Gage: C13062T5Gage CV HS 2010.docx (Added Nov 2, 2010)
Contact - Tom Gage: C13062T5TGage sea lion 2009 CV.doc (Added May 8, 2009)
Project Description - P16094T1Table 1. Samples and Procedures by Age Class.docx (Added Jul 14, 2011)
Project Description - P16094T1Table 2. Personnel experience.docx (Added Jul 14, 2011)
References - P16094T12ACUC.pdf (Added Feb 1, 2011)
References - P16094T12ACUC_Amendment.pdf (Added Feb 1, 2011)
References - P16094T12ADFG HS 1Jul11-30Jun13 NMFS Proposal FINALw SAC (4).docx (Added Jul 15, 2011)
References - P16094T12Literature cited.docx (Added Jul 14, 2011)
References - P16094T12Recent Publications.doc (Added Feb 1, 2011)
Resources Needed - P16094T15Resources Needed to Accomplish Objectives.docx (Added Jul 14, 2011)

Status

Application Status: Application Complete
Date Submitted: May 27, 2011
Date Completed: July 20, 2011
FR Notice of Receipt Published: July 29, 2011 **Number:** 0648-XA599

Comment Period Closed: August 29, 2011 **Comments Received:** Yes **Comments Addressed:** Yes
Last Date Archived: March 13, 2014

• MMPA Research/Enhancement permit

Current Status: Issued **Status Date:** September 20, 2011
Section 7 Consultation: N/A
NEPA Analysis: Categorical Exclusion
Date Cleared by General Counsel: September 20, 2011
FR Notice of Issuance/Denial Published: October 4, 2011 **Notice Number:** 0648-XA599
Expire Date: December 31, 2016
Analyst Information:

1) Tammy Adams Phone: (301)427-8401
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2) Courtney Smith Phone: (301)427-8433
Email: courtney.smith@noaa.gov

Modification Requests

Modifications Requested

Number	Title	Description	Status	Date Submitted	Date Issued	Issued Version
1	Captive whisker growth and	Permit amendment request Permit amendment request This amendment has 3 parts: 1) Request for captive whisker study We (ADF&G) would like to conduct a non-invasive study using captive seals held at the ASLC in Seward, AK. ASLC staff has agreed to supply a letter of support. Previous studies using phocid whiskers have been conducted and it has been shown that whiskers contain a temporal map of stable isotopes which can be used to describe changes in diet over time. However, the timeline of whisker growth and replacement is not well documented. There is some evidence that suggests that whiskers on different parts of the muzzle may grow at different rates or be replaced at different times. In order to make the dietary stable isotope information more useful, whisker growth and replacement across the entire muzzle needs to be examined. We propose to dye harbor seal whiskers with hair dye, and measure the growth, replacement and abrasion via photogrammetery.				

	growth and replacement study at ASLC	For more details see attached document. 2) Request to change the whisker collection on wild seals from 1/animal to 2/animal. Recent work has shown that harbor seal whiskers contain traces of hormones. Therefore analysis of whiskers will allow us to examine seasonal changes in stress (cortisol) and look at pregnancy rates and possibly pup survival based progesterone levels during pregnancy and lactation. Therefore, we would like to take one whisker for dietary stable isotopes and a second for hormone analysis. We would take the longest whisker from the posterior portion of the muzzle (one from each side). 3) Request to add photogrammetry to our wild seal takes We are interested in looking at the potential to divide individual harbor seals into age classes based on claw bands. Also, we are interested in documenting the whisker lengths on the muzzle of wild caught seals. Both of these would be accomplished by taking photographs of the fore-flippers and muzzle while the animal is sedated and restrained For questions, please contact Shawna Karpovich phone (907)459-7239 email shawna.karpovich@alaska.gov	Issued	11/17/2011	06/01/2012	P16094T14Mod1.pdf
2	add an authorized recipient to Appendix 2	Please add the following to Appendix 2, authorized recipient table: Authorized Recipient: University of Miami, Department of Pathology,Miami, FL, USA Sample Type: blood and swab samples Disposition: disease testing	Issued	03/29/2012	06/01/2012	P16094T14Mod2.pdf
3	Add a Co-Investigator	We request that Lauri Jemison be added as a Co-Investigator to this permit.	Issued	06/06/2012	06/14/2012	P16094T14Mod3.pdf
4	we request permission to apply dye to whiskers using a bag and to apply paint with a paint marker	During the first attempt to dye the whiskers, we painted the dye onto the whiskers, the dye is a gel and adhered to the fur on the muzzle very well, but did not stick to the whiskers because they are separate and do not have other hair or other whiskers near to cause the dye to stick via surface tension. We believe that we need to add something that will allow the whiskers to soak in the dye and propose to place a foam ring that has a bag affixed to it onto each side of the seals snout. We will then put the dye into the bag and squeeze out any air, this way the whiskers will be in contact with the dye during the entire dyeing period. The foam ring will be pressed onto the muzzle using tape, Velcro or handlers hands. This will not increase the amount of time that the skin is in contact with the dye because the same timing will be used as the dyeing attempt without the bag and during that application the dye pooled at the base of the whiskers and was held next to the skin via surface tension between the closely placed hairs on the muzzle causing full skin contact during the first attempt. The only difference will be that amount of time that the whiskers are in contact with the dye. We would also like to try using paint pens to place a paint mark on the whiskers. This will require manually marking the whiskers of interest (approximately 10 per side), with a marker that dispenses paint. We propose to paint on a band, not to paint the entire whisker. This will be done while the animal is sedated, and if successful may be used in place of the dye application methods described above. The addition of a bag apparatus to contain the dye, or manually marking with a paint pen, will occur only after the animals are sedated, so any additional stress from this procedure will be minimized.	Withdrawn	01/28/2013		

5	alternative methods to mark whiskers that are not hair dye	For our study examining the timing of harbor seal whisker molt and growth rates, we propose to test a collection of whisker marking methods. Each different method will be applied once, and tracked over time. The marks that persist for the longest will be chosen and all other methods will be abandoned. We would like to test this during the month of February so the best method can be employed when the study begins on April 1st. As with the dyeing protocol already in place, the animals will be sedated prior to handling, sedation will greatly minimize the stress associated with the proposed procedures. I will not go into the sedation details again because we do not intend to alter the protocol. However, in lieu of hair dye, we would like to try alternate methods to mark the whiskers. We will choose only 10-15 whiskers on each side of the muzzle to be marked. We will place 2-4 marks (bands of color, beads or metal bands) along the whisker shaft. This will allow measurements to continue even if one or two marks come off before the next marking session. If we observe a specific location (i.e. closest to the base) to be less likely to wear off, we will decrease the marks to 2-3/whisker. Because the marks are likely to be less persistent than permanent hair dye, we would like to alter the frequency, we currently have "Dye will be reapplied as needed, but not more than once in a 20 day period and not more than 8 times per year" in our proposal, we would like to change this to "Marks will be reapplied as needed, but not more than once in a 20 day period and not more than 12 times per year". Also, the currently approved amendment says, "Restraint will last approximately 30-60 minutes for dye applications". We would like to change that to "Restraint will last approximately 15-60 minutes for application of markers", because we believe that the marks may be applied in a shorter timeframe than the dye required. Proposed whisker marking media to be used: 1) nail polish 2) non-toxic paint 3) cyanoacrylate glue 4) plastic beads 5) metal crimping bands used in beading A. Danger of each marking component if accidentally ingested: 1) nail polish – some nail polishes contain phthalates which have been shown to be toxic causing decreased reproduction enlarged liver, and enlarged kidneys in rats http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1469857/pdf/envhper00314-0102.pdf. However, in this study, the rats were fed differing levels of phthalates in their diet for 14 consecutive days and the lowest concentration to show negative effects was at the 0.1% of diet level. Tongass, one of the captive harbor seals at ASLC, eats 1.58 kg of food/day. To reach the lowest level where negative effects were detected in the rat study, Tongass would have to accidentally ingest 1.58 grams of pure phthalates daily for 14 consecutive days. On average nail polish is 5% phthalate, so that would represent accidental consumption of 31.6 grams of nail polish, approximately equivalent to 6 or 7 bottles of nail polish per day for 14 consecutive days. We intend to only use a very small fraction of this amount, and only once/month. According to our protocol, it would be impossible for any of the harbor seals in this study to accidentally ingest enough nail polish for toxic phthalates to even approach 0.1% of their diet. 2) non-toxic paint – Because it is non-toxic accidental ingestion will not be dangerous. Also, similar to the nail polish, we propose to place a few marks on only a subset of the whiskers, so the overall quantity of paint used will be very small. 3) cyanoacrylate glue – This is Krazy or super glue and is non-toxic. Accidental ingestion of the glue after it is dried would pass through the digestive tract without issue. Accidental ingestion of the glue before it is dry would cause tissues to potentially stick together, however it reacts with water and becomes inert, so it would likely cause little adherence in the moist environment of the mouth. Furthermore, for ease of use we will chose varieties with the fastest drying time, and similar to the polish and paint will only be applying very small quantities, so the risk of ingesting any glue before it dries is minimal. 4) plastic beads – The beads will be very small, and only a few will be used. If accidentally ingested, these will pass through the digestive tract without issue. 5) metal crimping bands used in beading – The bands will be very small, and only a few will be used. If accidentally ingested, these will pass through the digestive tract without issue.	Issued	02/01/2013	03/05/2013	P16094T14Mod5.pdf

		B. Danger of inhaling fumes from each marking component: 1) nail polish – some nail polishes contain phthalates and fumes from wet nail polish can cause exposure to this toxin. A study was conducted to examine phthalate exposure to women who used 4 cosmetics (nail polish, perfume, hair product and deodorant) all of which contain phthalates on a daily basis. http://chemww2.rutgers.edu/~kyc/pdf/491/wilson/cosmetics2.pdf. This study concluded that even when using all 4 cosmetics 1-3 times on a daily basis, the exposure to phthalates was far below the minimal risk levels set by The Scientific Committee on Toxicity, Ecotoxicity and the Environment, The Agency for Toxic Substances and Disease Registry, and the International Programme on Chemical Safety. Exposure to only one phthalate containing product instead of 4, and monthly instead of daily will most certainly be below the minimal risk levels identified by the above mentioned agencies. An ointment will be placed into the eyes prior to marking to further protect them from fumes. 2) non-toxic paint – This does not have noxious fumes. 3) cyanoacrylate glue – The fumes from cyanoacrylate glue may cause mucosal membrane irritation. These fumes are immediately polymerized by the moisture in the membranes and become inert. These risks can be minimized by using small quantities of the glue and using it in well ventilated areas. The United States National Toxicology Program and the United Kingdom Health and Safety Executive have concluded that the use of ethyl cyanoacrylate is safe and that additional study is unnecessary. In this study, we will use very small quantities of glue and will be in a ventilated area. An ointment will be placed into the eyes prior to marking to provide additional protection. 4) plastic beads – no fumes associated. 5) metal crimping bands used in beading – no fumes associated. C. Danger of skin irritation from each marking component: 1) nail polish – some nail polishes contain phthalates and skin contact can cause exposure to this toxin. I have presented two studies above that examined the level of phthalates required to cause ill effects and have made a case that the quantities of nail polish that we propose to use are very small in comparison to the quantities required to cause problems associated with phthalate exposure. Furthermore, we will be marking the whisker, there may be some incidental contact with the skin, but skin exposure will be minimal. An ointment will be placed into the eyes prior to marking to further protect them from exposure. 2) non-toxic paint – This is not a skin irritant. 3) cyanoacrylate glue – These, in rare cases, may cause skin irritation. However, the glues were originally formulated to act as suture material and are generally considered safe. The United States National Toxicology Program and the United Kingdom Health and Safety Executive have concluded that the use of ethyl cyanoacrylate is safe and that additional study is unnecessary. We do not propose to use the glue on the skin and will attempt to avoid skin contact. An ointment will be placed into the eyes prior to marking to provide additional protection. 4) plastic beads – This is not a skin irritant. 5) metal crimping bands used in beading – This is not a skin irritant.			
6	Add Heidi Pearson as Co-Investigator	We request that Heidi Pearson be added as a Co-Investigator to this permit. Heidi will be conducting research in Berner's Bay, SE Alaska. She will conduct both aerial and vessel surveys to document harbor seal distribution and behaviors.	Issued	05/15/2013	03/07/2014
7	Request for approval of additional Authorized Recipient of samples	We are requesting permission for the Mystic Aquarium to be an authorized recipient of harbor seal (Phoca vitulina) blood samples for diagnostic testing of disease. Mystic Aquarium 55 Coogan Blvd. Mystic, CT 06355 USA	Withdrawn	06/05/2013	

Reports

Reports Required

Nbr	Report Type	Report Period		Date Due	Status	Date Received
		Start Date	End Date			
1	Annual	01/01/2012	12/31/2012	04/01/2013	Submitted	04/01/2013
2	Annual	01/01/2013	12/31/2013	04/01/2014	Submitted	03/12/2014
3	Annual	01/01/2014	12/31/2014	04/01/2015	N/A	
4	Annual	01/01/2015	12/31/2015	04/01/2016	N/A	
5	Annual	01/01/2016	12/31/2016	04/01/2017	N/A	
6	Final	01/01/2012	12/31/2016	07/01/2017	N/A	